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Following is translation of an article by Hans Beyer and Theodor Pyl in the German-Language Journal Chemische Berichte (Chemical Reports) Vol. 87, 1954, No. 10, pp 1505-1511.7

ON THEAZOLES

24th Communication: On C,N-diphenyl-N'-thiazolyl-(2)-formazane and its Tetrazolium Salts (*)

The preparation of C,N-diphenyl-N'-thiazolyl-(2)-formazanes which are substituted in the 4th and/or 5th position of the thiazolyl ring, is effected either by coupling of diazotized aniline with benzaldehyde-thiazolyl-(2)-hydrazone or by coupling of diazotized 2-amino-thiazoles with benzaldehyde-phenyl hydrazones. The second procedure presupposes the diazotability of the substituted 2-amino-thiazoles.

The individual formazanes can be converted through cyclodehydration by means of N-bromo succinimide into the corresponding tetrazolium salts used as indicators for biological reduction processes, and light-proof in contrast to presently known indicators.

The action of isoamyl nitrite on C,N-diphenyl-N'4-phenyl-thiazolyl-(2)7-formazane does not produce a tetrazolium salt but a nitroso-compound.

The formazanes discovered by H. von Pechmann (1) and E. Bamberger (2) independently of each other as well as the tetrazolium salts produced from them through cyclodehydration and the importance of the latter as reduction indicators and vital stains have been summarized by W. Ried (3). Sometime ago, Ried, Gick and Oertel (4) have described the preparation of C,N-diphenyl-N'-thiazolyl-(2)-formazane from benzaldehyde-phenyl-hydrazone and diazotized-2-amino-thiazole. Since we have been occupied, in connection with

(* Note: 23rd Communication: H. Beyer and H. Drews, Chemical Reports, Vol. 87, page 1500, 1954 (above); cf. Th. Pyl, Dissertation, Greifswalde, 1954.)

the investigation of thiazolyl-(2)-hydrazone (5), for sometime also with the synthesis of substituted N'-thiazolyl-(2)-formazanes and N,N'-dithiazolyl-(2)-formazanes as well as their tetrazolium salts, we are here publishing our first findings.

Starting from the benzaldehyde-thiazolyl-(2)-hydrazone described in the 12th (5) and the 15th (6) Communication, some of which contained in the 4th and/or 5th position of the thiazole nucleus methyl or phenyl groups, we obtain, by coupling them with phenyl diazonium acetate in pyridine methanol (1:1), the following formazanes: C₆N-diphenyl-N'-thiazolyl-(2)-formazane (I-a); the corresponding N'-[4-methyl-thiazolyl-(2)] (I-b); N'-[4-phenyl-thiazolyl-(2)] (I-c); and N'-[4,5-diphenyl-thiazolyl-(2)]-formazane (I-d). These crystallize from acetone in black needles with a greenish or bluish surface sheen. They dissolve in organic solvents with an intensively blue-violet color and form complex compounds (7) of intense color with the addition of Cu²⁺ or Co²⁺.

On the other hand, it should have been possible to obtain the same formazanes also through coupling of benzaldehyde-phenyl hydrazone with diazotized-2-amino-thiazoles, provided that the 2-amino-thiazoles substituted in the 4th and/or 5th position can be diazotized in the standard manner. 2-amino-, 2-amino-5-methyl- and 2-amino-4,5-dimethyl-thiazole prove to be relatively easily diazotable. By contrast, only very little formazane I-b was obtained from 2-amino-4-methyl-thiazole and experimentation with 2-amino-4-phenyl-thiazole was unsuccessful. This variant behavior of 2-amino-4-methyl- and 2-amino-4-phenyl-thiazole in "diazotization" has already been described in the preceding communication (*). Attempts at diazotization for 2-amino-4,5-diphenyl-thiazole were unsuccessful because of the low basicity of this compound. Through the second procedure indicated above, it was therefore possible only to prepare the following formazanes: C₆N-diphenyl-N'-thiazolyl-(2)-formazane (I-a), the corresponding -N'-[4-methyl-thiazolyl-(2)] (I-b); -N'-[5-methyl-thiazolyl-(2)] (I-e); and -N'-[4,5-dimethyl-thiazolyl-(2)]-formazane (I-f).

The identity of the formazanes (I-a and I-b) prepared in different ways, is explained by the formation of a hydrogen bridge between the hydrazone and the azo groups which involves an intramolecular bonding equilibrium in the formazyl group (8). Accordingly, the same formazane is also created by an exchange of the substituents in the N- and N'-atom. Cyclic dehydration of the above formazane to the corresponding tetrazolium salts was carried out initially with isoamyl nitrite in acetic acid. However, when treating the

yellowish-brown solutions thus obtained with 4 times the volume of ether, there were not precipitated the expected acetates but the free bases. Only after dissolving the bases in methanol bromo-hydracid, is it possible to precipitate the tetrazolium bromides with ether; depending on the basicity of the thiazole derivate, occasionally there becomes attached here 1 additional mol of hydrogen bromide which produces tetrazolium dihydribromide. The relatively insoluble picrates serve for further characterization of the salts.

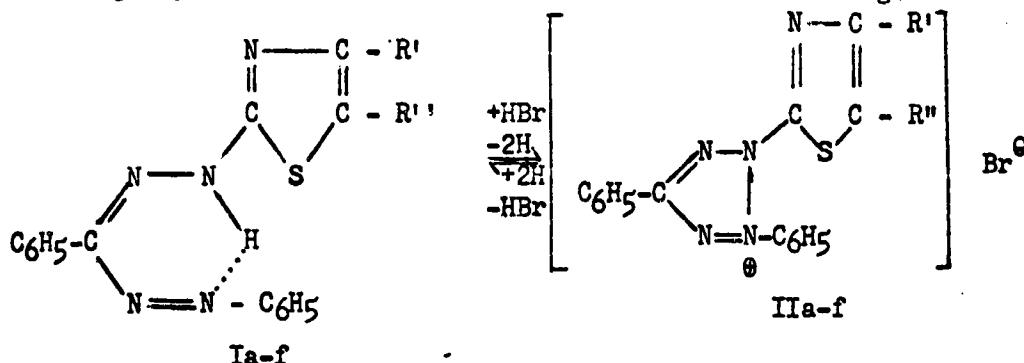
It was thus possible to obtain, from the formazane I-a already produced by Ried et al (4), the C,N-diphenyl-N'-thiazolyl-(2)-tetrazolium base (II-a) and/or the -tetrazolium dihydribromide in crystalline form.

The method of dehydration with isoamyl nitrite, however, was a failure for I-b and I-c as well as for the formazanes where the thiazole component also reacted in another manner under diazotization. When treating a solution of I-c in acetic acid with isoamyl nitrite, there was precipitated, even without the addition of ether, quantitatively a water-insoluble compound crystallizing in red needles which could not be reduced to the original formazane by sodium dithionite. Analysis showed that a nitroso group had entered the molecule. Analogous to the nitrosification of 2-amino-4-phenyl-thiazole described in the preceding 23rd communication (*), I-c is probably also nitrosified in the 5th position of the thiazole nucleus. Since the nitroso compound is soluble in lyes, it must have the constitution of an isonitroso-thiazolone derivative III so that simultaneously the formazane structure is broken up.

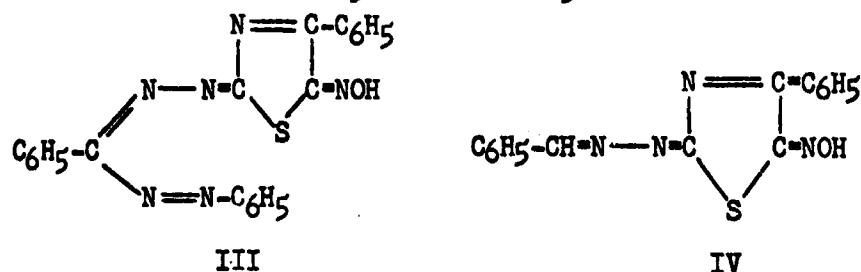
For further clarification of this question, we have treated as a simpler example benzaldehyde- $\overline{4}$ -phenyl-thiazolyl-(2)-hydrazone in the same way with isoamyl nitrite and obtained an easily crystallizing, yellow, very stable nitroso compound. The latter is also soluble in alkalis and the potassium salt is precipitated under cold in thin red needles. From the fact that this 5-nitroso compound did not furnish any formazane when coupled with diazotized aniline, we may conclude on the existence of the isonitroso-thiazolyl structure IV because coupling always starts in the NH-group of the hydrazone (3) which no longer exists in IV.

After it had been thus shown that isoamyl nitrite cannot always be employed for cyclodehydration of thiazolyl-substituted formazanes, we utilized N-bromosuccinimide in ethyl acetate according to R. Kuhn and W. Münzing (9). We were thus able to convert the formazanes I-a to I-f into the corresponding slight to intense

yellow tetrazolium bromides II-a II-f and/or their hydrobromides. The solubility in water amounts to 1% max. but decreases with increasing magnitude of the substituents in the thiazole ring.



a: R' = R'' = H, b: R' = CH₃, c: R' = C₆H₅, R'' = H, d: R' = R'' = C₆H₅, e: R' = H, R'' = CH₃, f: R' = R'' = CH₃



We further investigated in detail whether the above tetrazolium salts are capable of being used as test substances of biological reduction processes. They seem especially suitable for the following reasons:

1. The intensity of color of the N'-thiazolyl-(2)-formazane is greater than that of C,N,N'-triphenyl-formazane. The extinction coefficients of equimolecular solutions have ratios of 4:1 to 5:1. The staining produced in colibacteria was a deep black under the microscope (10).
2. The N'-thiazolyl-tetrazolium bromides are insensitive to light as compared to triphenyl-tetrazolium chloride (TTC). For comparison, 0.1% solutions of TTC and II-e treated with sodium bicarbonate were absorbed on filter paper and exposed to sunlight after drying. Whereas red triphenyl formazane was formed immediately in the first case, the paper saturated

with II-e did not assume any color. In this case, no disproportioning of the tetrazolium salt can take place (11).

The investigations on thiazolyl-(2)-formazane and -diformazane are being continued.

Description of Experimentation

C₁₆H₁₃N₅S-diphenyl-N'-thiazolyl-(2)-formazane (I-a):

a) 4 g Benzaldehyde-thiazolyl-(2)-hydrazone (1/50 mol) are dissolved in 50 ccm pyridine and 100 ccm methanol. A solution of phenyl diazonium acetate produced from 1.9 g aniline (1/50 mol) and buffered with sodium acetate is added at once under external cooling (sodium chloride and ice) to the foregoing solution already cooled to -5°. The internal temperature rises to 5-7° (but must not exceed 10°). This is stirred for 15 minutes with the addition of 100 ccm water and then left in the refrigerator for 2-3 hours. The amorphous black product is re-crystallized twice out of a large amount of boiling acetone. We obtain black needles with green surface sheen which melt at 137°; yield 3.8 g (66% of the theoretical amount).

C₁₆H₁₃N₅S (307.4); calculated N 22.79; found N. 23.07

b) The same formazane is obtained from coupling benzaldehyde-phenyl hydrazone with diazotized (2)-amino-thiazole. Fusion point 137°. The fusion point of 158-159° indicated by Ried et al (3) could not be reached, in spite of repeated re-crystallization by us.

C₁₆H₁₃N₅S-diphenyl-N'-thiazolyl-(2)-tetrazolium base:

0.3 g I-a are mixed drop by drop with isoamyl nitrite in 6 ccm acetic acid until the intense color of the formazane has disappeared. From the yellowish-brown solution, the base is precipitated by the addition of 30 ccm of ether in the form thin yellowish prisms which melt after re-crystallization from very little methanol or precipitation from acetic acid/ether at 203°. Yield 0.24 g (74% of theoretical amount).

C₁₆H₁₃ON₅S (323.4); calculated N 21.66; found N 21.65

If the base is dissolved in methanol and treated with an alcoholic solution of picric acid, the picrate is precipitated in yellow needles. Fusion point 108°.

C₁₆H₁₂N₅S·C₆H₃O₇N₃ (535.5); Calculated N 20.93; Found N 21.23

C₁₁N-diphenyl-N'-thiazolyl-(2)-tetrazolium-bromide-hydrobromide
(II-a):

0.62 g of I-a (1/500 mol) are dissolved in 20 ccm of ethyl acetate with the addition of a solution of 0.36 g N-bromosuccinimide (1/500 mol) in 20 ccm of ethyl acetate. After adding a few drops of concentrated hydrobromic acid, this precipitates thin yellow prisms which are washed in ice-cold ethyl acetate. After re-crystallization from a great deal of 1-% methanol hydrobromic acid, the substance melts at 149°. Yield 0.8 g (85% of theoretical amount).

C₁₆H₁₂N₅BrS·HBr (467.2); Calculated N 14.99; Found N 15.36

The same produce is obtained from the above tetrazolium base in hot alcohol with hydrobromic acid.

C₁₁N-diphenyl-N'-/4-methyl-thiazolyl-(2)-7-formazane (I-b):

a) 4.4 g benzaldehyde-4-methyl-thiazolyl-(2)-7-hydrazone (1/50 mol) are dissolved in 50 ccm of pyridine and 100 ccm of methanol and, as described above, mixed with a solution of phenyl diazonium salts prepared from 1.9 g aniline (1/50 mol). Processing is effected in the same manner. Thin black needles are obtained from acetone. Fusion point 166°. Yield 3.2 g (50% of thiazolyl).

C₁₇H₁₅N₅S (321.4); Calculated N 21.80; Found N 21.95

b) 2.3 g 2-amino-4-methyl-thiazole (1/50 mol) are dissolved in 40 ccm of 4nHCl and diazotized in the usual manner with 1.4 g sodium nitrite. A solution of 3.5 g benzaldehydophenyl hydrazone (1/50 mol) in 300 ccm of alcohol and 20 g of sodium hydroxide is added to the diazonium-salt solution previously cooled to -10°. After separation of the tar products, the red solution is highly concentrated over the water bath and extracted with chloroform after cooling. After evaporation to about 3 ccm of chloroform, the solution is treated with 3 times the amount of methanol. After being left to stand for several days, small black needles are precipitated. Fusion point 166°. Yield 1-3% of theoretical amount.

C₁₁N-diphenyl-N'-/4-methyl-thiazolyl-(2)-7-tetrazolium bromide (II-b):

0.32 g of I-b (1/1,000 mol) are mixed in 10 ccm of ethyl acetate with a solution of 0.2 gram N-bromo succinimide in 10 ccm ethyl acetate which immediately precipitates orange-colored prisms which melt after re-crystallization from a small amount of methanol

at 117°. Yield 0.3 g (60% of theoretical amount).

$C_{17}H_{14}N_5BrS$ (400.3); Calculated N 17.49; Found N 17.26

C_6N -diphenyl- N^1 - $\bar{4}$ -phenyl-thiazolyl-(2) $\bar{7}$ -formazane (I-c):

5.6 g benzaldehyde- $\bar{4}$ -phenyl-thiazolyl-(2) $\bar{7}$ -hydrazone (1/50 mol) are dissolved under heat in 50 ccm pyridine and 100 ccm methanol and after cooling to -5°, mixed with 1/50 mol phenyl diazonium acetate as above. After 24 hours, about 4-5 g of raw product has been precipitated. This is dissolved in 60 ccm of boiling pyridine, filtered, mixed with 5 ccm of hot water and left to cool slowly. On the following day, the crystalline precipitate is drawn off and recrystallized from acetone. We obtain black needles with a greenish sheen. Fusion point 189-190°. Yield 2.8 g (38% of theoretical amount).

$C_{22}H_{17}N_5S$ (383.4); Calculated N 18.27; Found N 18.59

C_6N -diphenyl- N^1 - $\bar{4}$ -phenyl-thiazolyl-(2) $\bar{7}$ -tetrazolium bromide (II-C):

0.4 g of I-c (1/1,000 mol) are mixed in 10 ccm ethyl acetate with 0.2 g N-bromosuccinimide (1/1,000 mol) in 10 ccm ethyl acetate. Immediate discoloration and precipitation of orange-colored prisms which melt at 172° after re-crystallization from methanol.

$C_{22}H_{16}N_5BrS$ (462.3); Calculated N 15.15; Found N 15.07

C_6N -diphenyl- N^1 - $\bar{5}$ -nitroso- $\bar{4}$ -phenyl-thiazolyl-(2) $\bar{7}$ -formazane (III):

0.4 g of I-c (1/1,000 mol) are mixed in 20 ccm acetic acid with isoamyl nitrite drop by drop until discoloration. Red-orange colored needles are precipitated which melt at 174° (decomposition?) after re-crystallization from acetone. The substance dissolves with a red color in diluted lyes.

$C_{22}H_{16}ON_6S$ (412.4); Calculated N 20.38; Found N 20.34

N -Benzaldehyde- N^1 - $\bar{5}$ -isonitroso- $\bar{4}$ -phenyl-thiazolone-(2) $\bar{7}$ -azine (IV):

5.6 g benzaldehyde- $\bar{4}$ -phenyl-thiazolyl-(2) $\bar{7}$ -hydrazone (1/50 mol) are mixed in 250 ccm of alcohol and 5 ccm of 4nHCl at room temperature with a solution of 1.5 g sodium nitrite under stirring. The yellow needles precipitated are re-crystallized out of a large amount of acetone. Fusion point 208°(decomposition?).

$C_{16}H_{12}ON_4S$ (308.3); Calculated N 18.17; Found N 18.01

The azine dissolves easily in a 5% solution of hot potassium hydroxide; when cooling, the potassium salt crystallizes into long orange-colored needles which crumble rapidly on exposure to air with the absorption of carbon dioxide. If the aqueous solution of the potassium salt is mixed with a few drops of a silver nitrate solution, the non-soluble silver salt is precipitated in small scarlet-red needles.

Monoacetyl compound of IV:

1 g of IV are heated in 10 ccm acetone hydride and red-orange colored rhombi are isolated. Fusion point 211°.

$C_{18}H_{14}O_2N_4S$ (350.3); Calculated N 16.00; Found N 16.37

C_6N -diphenyl-N¹-[4,5-diphenyl-thiazolyl-(2)]-formazane-(I-d):

7 g benzaldehyde-[4,5-diphenyl-thiazolyl-(2)]-hydrazone (1/50 mol) are dissolved in 100 ccm each of pyridine and methanol and mixed as above at -5° with a solution of phenyl diazonium acetate prepared from 1.9 g aniline. Initially a tar-like product is precipitated which solidifies after a few hours. It is dissolved in a little chloroform, evaporated, and mixed with 50 ccm of alcohol. After 24 hours in the refrigerator, black needles with a blue sheen are precipitated. Fusion point 172-174°. Yield 1.8 g (22% of theoretical amount).

Better yields are furnished by the following processing of the raw product: It is dissolved in 50 ccm of acetic acid and mixed drop by drop with isooamyl nitrite until the violet color has disappeared. Four volumes of ether are added to the yellowish-brown solution when the tetrazolium salt II-d is precipitated in yellow prisms. This is dissolved in acetone and reduced to formazane I-d with a calculated amount of sodium dithionite. Yield 60-70% of theoretical amount.

$C_{28}H_{21}N_5S$ (459.5); Calculated N 15.24; Found N 15.69

C_6N -diphenyl-N¹-[4,5-diphenyl-thiazolyl-(2)]-tetrazolium-bromide (I-d):

0.5 g of I-d are mixed in acetic acid as above with 0.2 g N-bromosuccinimide. Upon addition of an alcoholic solution of picric acid, the picrate is precipitated in orange-colored needles.

$C_{28}H_{20}N_5S \cdot C_6H_3O_7N_3$ (687.6); Calculated N 16.30; Found N 16.00

C₁₁N-diphenyl-N'-[5-methyl-thiazolyl-(2)]-formazane (I-e):

4.7 g 2-amino-5-methyl-thiazole (1/25 mol) are dissolved in 30 ccm of 5nHCl and diazotized as customary with 3 g sodium nitrite. The diazonium salt solution is mixed at -10° with a solution of 6 g benzaldehyde-phenyl hydrazone (1/25 mol) in 100 ccm of pyridine and 100 ccm of methanol with the addition of 10 g sodium acetate under stirring. After 10 minutes, 100 ccm of water are added and the precipitate created is drawn off after two hours. The raw product is re-crystallized from a large amount of acetone. It forms black needles with a greenish sheen. Fusion point 160°. Yield 6.4 g (68% of theoretical amount).

C₁₇H₁₅N₅S (321.4); Calculated N 21.80; Found N 21.91

C₁₁N-diphenyl-N'-[5-methyl-thiazolyl-(2)]-tetrazolium-dihydrobromide (II-e):

0.65 g of I-e (1/500 mol) are dissolved in 10 ccm of ethyl acetate under slight heat and 0.36 g N-bromosuccinimide in 15 ccm of ethyl acetate are added to the blue-violet solution which produces immediately strong discoloration. Upon adding of concentrated hydrochloric acid, faintly yellow needles become crystallized. Fusion point 178°. Yield 0.7 g (87% of theoretical amount).

C₁₇H₁₄N₅BrS·HBr (481.2); Calculated N. 14.56; Found N 14.75

C₁₁N-diphenyl-N'-[4,5-dimethyl-thiazolyl-(2)]-formazane (I-f):

4 g benzaldehyde-phenyl hydrazone are dissolved in 50 ccm pyridine and 100 ccm methanol with the addition of 10 g sodium acetate and mixed at -10° and under stirring with a solution of diazonium salt from 4 g 2-amino-4,5-dimethyl-thiazole-hydrobromide in 50 ccm of 4nHCl. After 15 minutes, 100 ccm of water are added and the precipitating raw product is left to stand for three hours at 0°. Re-crystallized from acetone, we obtain black needles with a blue sheen. Fusion point 164°. Yield 4.8 g (69% of theoretical amount).

C₁₈H₁₇N₅S (335.4); Calculated N 20.88; Found N 21.03

C₁₁N-diphenyl-N'-[4,5-dimethyl-thiazolyl-(2)]-tetrazolium-bromide (II-f):

0.35 g of I-f (1/1,000 mol) are mixed in ethyl acetate with

0.2 g N-bromo succinimide. We obtain yellow needles with a melting point of 171°. Yield 0.35 g (85% of theoretical amount).

$C_{18}H_{16}N_2BrS$ (414.3); Calculated N 16.91; Found N 16.94

Footnotes

- 1) Ber. dtsch. chem. Ges. 25, 3175 1892.
- 2) Ber. dtsch. chem. Ges. 25, 3201 1892.
- 3) Angew. Chem. 64, 391 1952.
- 4) Liebigs Amm. Chem. 581, 36 1953.
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- 8) I. Hausser, D. Jerchel u. R. Kuhn, Chem. Ber. 82, 515 1949; 84, 651 1951.
- 9) Chem. Ber. 86, 858 1953.
- 10) These investigations were carried out in the Epizootic Research Institute at the Island of Riems of the Friedrich-Loeffler Institute of the German Academy of Agricultural Sciences.
- 11) F. Weygand u. J. Frank, Z. Naturforsch. 3b, 377 1948; I Hausser, D. Jerchel u. R. Kuhn, Chem. Ber. 82, 195 1948.